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SEXUAL DIMORPHISM OF MEDIUM-SIZED NEURONS WITH SPINES IN HUMAN NUCLEUS ACCUMBENS

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Abstract - The nucleus accumbens is a limbic nucleus, representing part of the striatum body, and together with the caudate nucleus and putamen, it lies on the septum. The aim of this study was to examine morphological sexual dimorphism in spine density and also to undertake an immunohistochemical study of expression for estrogen and progesterone receptors in the medium-sized neurons in the nucleus accumbens. The research was conducted on twenty human brains of persons of both sexes, between the age of 20-75 years. The Golgi method was applied to determine the types and subtypes of neurons, morphologies of soma, dendrites and axons, as well as the relations between the cells and glial elements. The following were quantitatively examined: the maximum diameter of the neurons, the minimal diameter of the neurons, and the total length of the dendrites. The expression of receptors for estrogen and progesterone, their distribution and intensity were defined immunohistochemically. The parameters of the bodies of neurons in the shell and core of the nucleus accumbens were studied in both men and women. No statistically significant differences were found. Examination of the spine density showed statistical significance in terms of a higher density of spines in women. Immunohistochemically, in the female brain estrogen expression is diffusely spread in a large number of neurons; it is extra nuclear, of granular appearance and high intensity. In the male brain, expression of estrogen is visible and distributed over about one half of different types of neurons; it is extra nuclear, of granular appearance, mostly of middle and low staining intensity. Expression of progesterone in the female brain was very discreet and on a very small number of neurons; it was extra nuclear and with a weak staining intensity. Expression of progesterone in the male brain was distributed on a small number of neurons. It had a granular appearance, it was extra nuclear, with a very low staining intensity. Our results show differences in the morphology as well as expression of receptors for estrogen and progesterone on medium-sized neurons with spines in the nucleus accumbens of men and women.

Key words: Nucleus accumbens, neurons, Golgi, estrogen, progesterone

INTRODUCTION

The nucleus accumbens (NA), from the Latin word accumbere meaning sits on or leans on, is the rostral, ventromedial part of the striatum body, together with the caudate nucleus and putamen, lying on the septum. Ramon Cajal (1911) described a medium-sized neuron in the striatum, densely covered with spines,

which appeared in the region of dorsal striatum and considered as the main neuron in this region.

The nucleus accumbens, as part of the limbic system, is well known as a drug-related brain region. The nucleus accumbens has a key role in reward and enforcement of neuronal processes via glutaminergic afferent pathways originating from the basolateral amygdala, ventral subiculum and medial prefrontal cortex (Mc Donald, 1991). Stimulated dopamine transmission in the human nucleus accumbens is related to addiction and positive reinforcement of many drugs (Hoebel et al., 1989).

Medium-sized neurons with spines (MSN) represent a heterogeneous group of neurons that are characterized by a morphologically different form of soma, with a size of 10-20 μm in greatest diameter, and a variable number of dendrites (2-8). The presence of spines was not registered on the primary dendrites, while the secondary and tertiary dendrites were covered with spines of different densities. Such morphology of spines provides an enormous variability of contacts between dendrites, both within the nucleus and distant structures.

There are two possible biological mechanisms that mediate in sexual dimorphism. First, sex differences in the brain during development can produce independent sex differences in the brains of adults that persist independently from circulating gonadal hormones in adults. Second, in adults, circulating gonadal hormones may affect the brain system in the processes of addiction and reward-conditioned behavior.

In men, androgens during prenatal life permanently organize the nucleus of the limbic system, hypothalamus and spinal cord, forming a male phenotype (androgens in the same period in women form the female phenotype). The organizational effects of gonadal hormones include differences in the volume of specific brain nucleus, the number and/or spine density on the dendrites of neurons, complexity of the synaptic connections and the expression of neuropeptides (De Vries et al., 2002).

MATERIALS AND METHODS

Research was conducted on twenty human brains, belonging to persons of both sexes (male-to-female ratio equal 10:10), between 20-75 years of age. All brains were fixed after autopsy within ten hours from the time of death. The brains were fixed in phosphate-buffered 10% formalin dilutions (3.7% formaldehyde). The Golgi method was applied to determine the types of neurons, subtypes of neurons, morphologies of soma, dendrites and axons, as well as the relations between the cells, synapses, blood vessels and glial elements. The following were studied quantitatively: maximum diameter of neurons (D max), minimal diameter of neurons (D min), and total length of dendrites (TDL). TDL is a total dendritic length that is the sum of the lengths of all dendritic extensions of a neuron in microns. Using the parameters D max and D min and the formula:

$$(D_{max} \times D_{min2}) \times \pi/6,$$

we obtained the volume of a cell body. The spine densities were compared by, counting the spines on the secondary and tertiary dendrites along the entire length of the dendrites, and then calculating the average density at 10 μm. Quantitative studies were performed using a Zeiss Axiovision 3.0.6. program.

Immunohistochemical methods indicate the neurotransmitter and modulatory activities of individual neurons, as well as the morphological features of individual neurons, indicating the types of neurons, the shape and morphology of soma.

For immunohistochemical staining, we used a primary monoclonal anti-human mouse antibody that binds to the neurotransmitters contained in the soma or extensions of neurons. After addition of the primary antibody, the preparations were kept in a water bath for 60 min and then rinsed with PBS. A biotinylated anti-mouse secondary antibody, streptavidin-HRP (horseradish peroxidase) and DAB (3,3'-diaminobenzidine) were successively applied to the preparations, followed by rinsing in PBS after the use of each substances. The preparations were rinsed

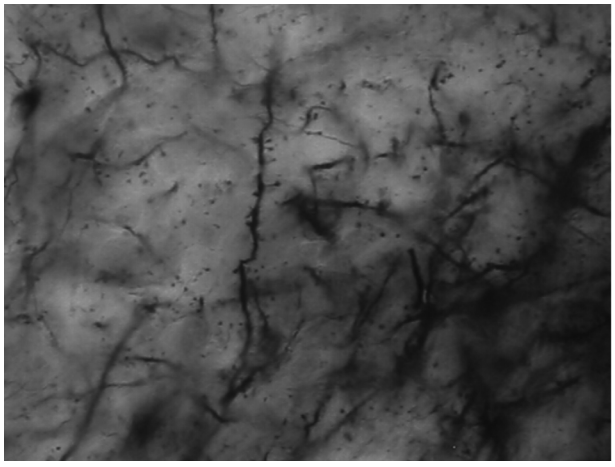

Fig. 1. Spine density on secondary dendrite, female sex, 400 X magnification

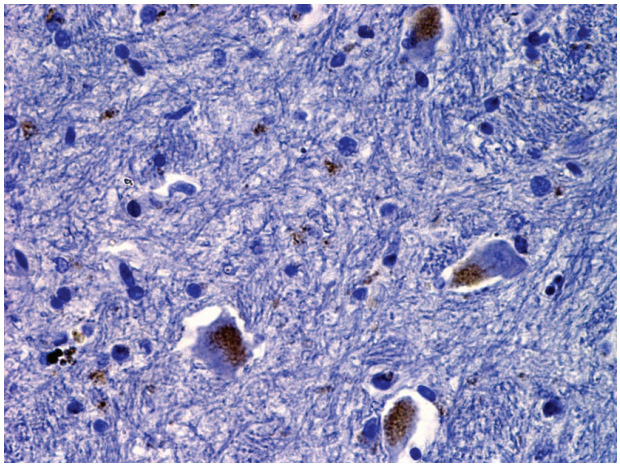

Fig. 2. Expression of estrogen (ERα) in the medium-sized neurons with spines in the nucleus accumbens, female sex, 400 X magnification

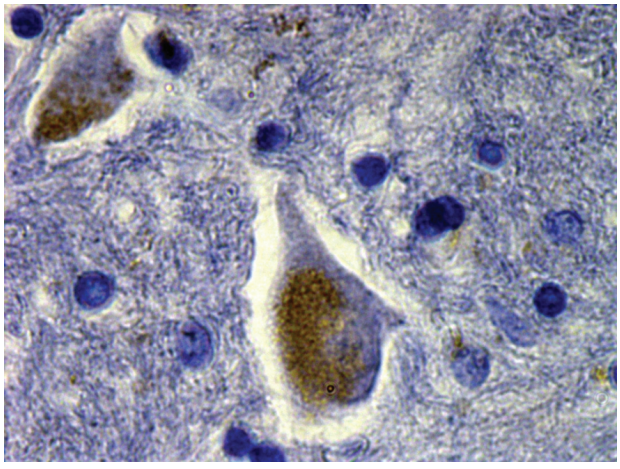

Fig. 3. Expression of estrogen (ERα) in the medium-sized neurons with spines in the nucleus accumbens, female sex, 640 X magnification

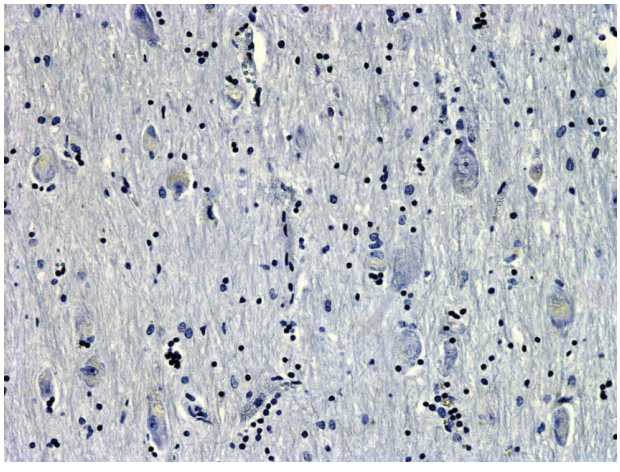

Fig. 4. Expression of estrogen (ERα) in the medium-sized neurons with spines in the nucleus accumbens, male sex, 200 X magnification

with distilled water, stained with hematoxylin and again rinsed with water. Further procedure involved the dehydration of the preparations by increasing the concentrations of alcohol.

RESULTS

Sexual dimorphism analysis

We examined if there were differences within certain sub-regions of the nucleus. We studied the parameters of the bodies of neurons (Dmax, Dmin, volume of the body of a neuron or a soma, Vol) in the shell of the nuclei (with the dominant types of the fusiform neurons and fusiform neurons with lateral dendrites of neurons), in both men and women. We found no statistically significant differences. The same parameters for neuron soma in the core of both sexes, where pyramidal-like neurons and multipolar neurons dominated, were studied, We found that there was no statistically significant difference.

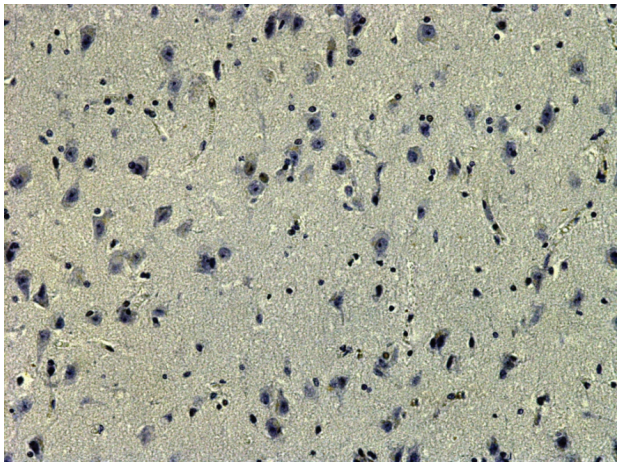

Fig. 5. Expression of progesterone in the medium-sized neurons with spines in the nucleus accumbens, female sex, 200 X magnification

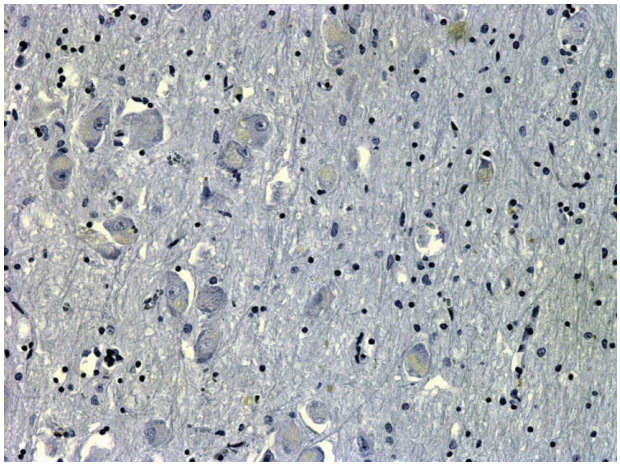

Fig. 6. Expression of progesterone in the medium-sized neurons with spines in the nucleus accumbens, male sex, 200 X magnification

After this, we examined the spine density, measuring them at a distance of 10 μm along the secondary and tertiary dendrites where their density was highest. In the shell spine, the density was 8.6 ± 0.5 in women, and 6.4 ± 0.8 in men (Table 1.) This result was statistically significant in terms of higher spine density in women. At the core, we registered 9.2 ± 0.9 in women compared to 6.9 ± 0.8 in men (Table 2). The result shows a statistically significant difference in terms of higher spine density in women than in men at both the level of individual sub-regions and across the entire nucleus (Fig. 1).

Sexual dimorphism, immunohistochemical expression of sex hormones

The neurons in the nucleus accumbens have receptors for estrogen and progesterone. They are extra nuclear, having different distributions and intensities in male and female brains. In the nucleus accumbens in the female brain, the expression of the estrogen receptors was diffuse and evident on a large number of different types of neurons. It was always extra nuclear, granular in appearance and of high intensity and occupying up to 2/3 of the soma (Figs. 2, 3).

In the nucleus accumbens in the male brain, the expression of estrogen receptors (ERα) was also evident. It was distributed in about half of different types of neurons; it was extra nuclear, granular in appearance and mostly having middle to low staining intensity (Fig. 4).

The expression of progesterone in medium-sized neurons with spines in the female brain was very discreet, present on a very small number of neurons; it was extra nuclear and with a low intensity (Fig. 5). The expression of progesterone in male brain neurons was distributed over a small number of neurons; it was extra nuclear and with very weak intensity (Fig. 6).

DISCUSSION

Hormonal changes can directly induce changes in the plasticity of spines secreted peripherally (taken exogenously) and can be metabolized locally through a variety of hormone receptors. It was hypothesized that hormone-dependent dendritic plasticity that leads to changes in dendritic architecture, depends on the effects of hormones. Gonadal hormones have powerful effects on brain structure. They control animal behavior.

Estrogen, with its clearly defined role in reproductive functions, performs various activities in many regions of the nervous system that affect high cognitive functions, pain mechanisms, fine motor skills,

Table 1. Morphological characteristics of shell medium-spiny neurons of nc. accumbens

shell	male	female	Σ
D max	21.59 ±3.64 μm	23.12 ±3.21 μm	n.s.
D min	8.76 ± 0.84μm	9.58 ± 0.72μm	n.s.
vol	866.25 ± 176.7 μм^3	1110.97 ± 126.7 μм^3	n.s.
spine density	6.4 ± 0.8	8.6 ± 0.5	p<0.01

D max – maximal diameter of soma, D min – minimal diameter of soma, vol – volume of soma

Table 2. Morphological characteristics of core medium-spiny neurons of nc. accumbens

core	male	female	Σ
D max	22.72 ± 1.24 μм	24.22 ± 1.54 μм	n.s.
D min	14.33 ± 2.26 μм	15.44 ± 2.13 μм	n.s.
vol	2529.79 ± 457.5 μм$^{3.}$	3133.47 ± 324.5 μм$^{3.}$	n.s.
spine density	6.9 ± 0.8	9.2 ± 0.9	p<0.01

D max – maximal diameter of soma, D min – minimal diameter of soma, vol – volume of soma

mood and susceptibility to seizures. Estrogen also has a neuroprotective role against stroke and Alzheimer's disease. Estrogen activity is achieved through two distinct intracellular estrogen receptors, Erα and Erβ, which are found in the nucleus of some neurons, and through some less typical mechanisms.

Estrogen causes a change in the dendritic plasticity of spines in the hippocampus, ventromedial nucleus and C1 pyramidal-like neurons of the dorsal hippocampus (Weiland et al., 1997). Estrogen most likely increases the density of spines in pyramidal-like neurons by indirect mechanisms.

Current literature indicates that female rats are more sensitive to cocaine addiction than male rats and may be more vulnerable to the powerful effects of psychostimulants (Chen and Kandel, 2002; Kosten, 1993). These studies have revealed different patterns of sexual behavior in response to cocaine at all stages of addiction, including initiation, maintenance and relapse (Lynch et al., 2002). A clearer picture that is emerging indicates the biological basis of sex-specific differences in addiction to cocaine, and that they are differently regulated in the CNS of male and female rats by gonadal hormones (Festa, and Quinones-Jenab, 2004).

When we examined the spine density, we noticed sexual dimorphism in both subregions (core and shell), and at the level of the entire nucleus accumbens in terms of higher density in females. Similar results were registered by Forlano and Wooley (2010) who noted a higher spine density in female rats at the core of the nucleus accumbens, as well as at the level of the whole nucleus, but not at the level of the shell. We have shown that the spine density was higher in women, thus the lower spine density in men suggests that dopamine has a smaller impact on the NA.

These differences are evident in the density of spines, especially on the distal (secondary and tertiary) dendrites of medium-sized neurons with spines. This tells us that early embryonic development defines the morphology of the neurons, and that later pubertal modulates these neurons by reward conditioned behavior (Forlano and Woolley, 2010).

After examining the synaptic activity of NAK and spine density, Wisman et al. (2011) concluded that the density of synapses is higher in female rats than in males and that it is higher in the core than in the shell of NAK. We registered a significantly higher expression of extra nuclear estrogen receptors

ERα, in the neurons of NAK in women than in men. As for the expression of extra nuclear progesterone receptors PR, in general, it is considerably less than the expression of ERα in both sexes. Similar gender differences, but on the other structures of the brain, were also obtained by other authors (Lenz et al., 2012; Quadros et al., 2002).

It is known that during embryonic development, testicular secretion leads to sexual dimorphism in brain organization (Breedlove and Jordan, 2001). There are also the effects of exposure to gonadal hormones during puberty that contribute to different brain development and behavior (Romeo and Sisk, 2001; McEwen, 2001). Estradiol is known as a hormone that affects many aspects of behavior, as well as in the processes of addiction (Pellis, 2002; Hu and Becker, 2003). In experimental animals, it was observed that high doses of estradiol enhances cocaine addiction (Robinson and Berridge, 2000).

Estradiol stimulates dopamine release in the ventral and dorsal striatum (nc. caudatus, putamen and nc. accumbens), increases dopamine takeover in NAK, influencing the density of receptors for dopamine (Lynch et al., 2001). The ability of estradiol to facilitate dopaminergic activity is considered key to enhancing the effects of cocaine on behavior in females (Jelks et al., 2007). Steroid hormones and their receptors are powerful regulators of gene transcription in the CNS and have the potential to change permanently the structure and function of the developing brain. In our study, we have shown sexual dimorphism in the expression of extra nuclear estrogen and progesterone receptors in the neurons of the human nucleus accumbens.

REFERENCES

Breedlove, S.M., and *C.L. Jordan* (2001) . The increasingly plastic, hormone-responsive adult brain. *Proc Natl Acad Sci USA*, **98**(6), 2956-7

Chen, K., and *D. Kandel* (2002). Relationship between extent of cocaine use and dependence among adolescents and adults in the United States; *Drug Alcohol Depend*., **68**(1), 65-85.

De Vries, G.J., Rissman, E.F., Simerly, R.B., Yang, L.Y., Scordalakes, E.M., Auger, C.J., Swain, A., Lovell-Badge, R., Burgoyne, P.S., and *A.P. Arnold* (2002). A model system for study of sex chromosome effects on sexually dimorphic neural and behavioral traits. *J Neurosci.* **22**(20), 9005-14.

Festa, E.D., and V. Quinones-Jenab (2004). Gonadal hormones provide the biological basis for sex differences in behavioral responses to cocaine; *Hormones and Behavior*, **46**, 509-519

Forlano, P.M., and *C.S. Woolley* (2010). Quantitative analysis of pre- and postsynaptic sex differences in the nucleus accumbens; *Comp Neurol*. **518**(8), 1330-1348.

Hoebel, B.G., Hernandez, L., Schwartz, D.H., Mark, G.P., and *G.A. Hunter* (1989). Microdialysis studies of brain norepinephrine, serotonin, and dopamine release during ingestive behavior, theoretical and clinical implications. *Ann NY Acad Sci,* **575**, 171-91.

Hu, M., and *J.B. Becker* (2003). Effects of sex and estrogen on behavioral sensitization to cocaine in rats; *J Neurosci.* **23**(2), 693-9.

Jelks, K.B., Wylie, R, Floyd, C.L., McAllister, A.K., and *P.J. Wise* (2007). Estradiol targets synaptic proteins to induce glutamatergic synapse formation in cultured hippocampal neurons: critical role of estrogen receptor-alpha; *Neurosci.* **27**(26), 6903-13.

Kosten, T.R. (1993) Clinical and research perspectives on cocaine abuse: the pharmacotherapy of cocaine abuse; *NIDA Res Monogr*. **135**, 48-56.

Lenz, K.M., Nugent, B.M., and *M.M. McCarthy* (2012). Sexual differentiation of the rodent brain: dogma and beyond; *Front Neurosci.,* **6**, 26.

Lynch, W.J., Roth, M.E., Mickelberg, J.L., and *M.E. Carroll* (2001). Role of estrogen in the acquisition of intravenously self-administered cocaine in female rats; *Pharmacol Biochem Behav.* **68**(4), 641-6.

Lynch, W.J., Roth, M.E., and *M.E.Carroll* (2002). Biological basis of sex differences in drug abuse: preclinical and clinical studies; *Psychopharmacology (Berl)*. **164**(2), 121-37.

Mc Donald, A.J. (1991). Topographical organization of amygdaloid projections to the caudoputamen, nc. accumbens, and related striatal-like areas of the rat brain. *Neuroscience,* **44**(1), 15-33.

McEwen, B.S. (2001) Invited review. Estrogens effects on the brain: multiple sites and molecular mechanisms. *J Appl Physiol.* **91**(6), 2785-801.

Pellis, S.M. (2002). Sex differences in play fighting revisited: traditional and nontraditional mechanisms of sexual differentiation in rats. *Arch Sex Behav.* **31**(1), 17-26.

Quadros, P.S., Pfau, J.L., Goldstein, A.Y., De Vries, G.J., and *C.K. Wagner* (2002). Sex differences in progesterone receptor

expression: a potential mechanism for estradiol-mediated sexual differentiation; *Endocrinology*. **143**(10), 3727-39.

Ramon y Cajal, S. (1911). *Histologie du Systeme Nerveux de l Homme et des Vertebres.* Marloine; Paris

Robinson, T.E., and *K.C., Berridge (*2000). The psychology and neurobiology of addiction: an incentive-sensitization view. *Addiction*. **95** Suppl 2:S91-117

Romeo, R.D., and *C.L.Sisk (*2001). Pubertal and seasonal plasticity in the amygdala. *Brain Res.* **889**(1-2):71-7.

Weiland, N.G., Orikasa, C., Hayash,i S., and *B.S. McEwen (*1997). Distribution and hormone regulation of estrogen receptor immunoreactive cells in the hippocampus of male and female rats. *J Comp Neurol.* **388**(4):603-12.

Wissman, A.M., May, R.M., and *C.S. Woolley (*2011). Ultrastructural analysis of sex differences in nucleus accumbens synaptic connectivity; *Brain Struct Funct*. **217**(2):181-90.